Edible Corn-zein-based Coating Incorporated with Nisin or Lemongrass Essential Oil Inhibits *Listeria monocytogenes* on Cultured Hybrid Striped Bass, *Morone chrysops* × *Morone saxatilis*, Fillets During Refrigerated and Frozen Storage

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Abstract

Listeria monocytogenes presents a serious threat to consumer safety because it is resistant to various food storage techniques, including reduced or modified atmosphere packaging, refrigerated storage, and increased salt concentration. Edible coatings incorporated with natural antimicrobials have been suggested to control pathogenic and spoilage bacteria on a variety of meat products. In this study, edible zein-based coatings incorporated with nisin and lemongrass essential oil (LGEO; 8%) were evaluated for antibacterial action against *L. monocytogenes* and spoilage organisms on fresh, cultured hybrid striped bass, *Morone saxatilis* × *Morone chrysops*, under two storage conditions (refrigerated or frozen) and two packaging types (polyvinyl chloride [PVC] and vacuum packing) over time. Corn-zein-based edible coatings were found to be an effective carrier for nisin and LGEO. Fillets coated with nisin showed the largest decrease in *L. monocytogenes* cell counts in both PVC and vacuum-packaged samples in both refrigerated and frozen product, while fillets coated with LGEO showed intermediate inhibition of *L. monocytogenes* cell counts, with the strongest LGEO antibacterial effect being found in frozen product regardless of packaging. Both nisin and LGEO treatments were most effective in PVC-packaged fillets compared to vacuum-packaged fillets, but the difference in bacterial loads between packaging methods was minor. Bacterial loads on refrigerated product tended to increase slightly after 5-d storage regardless of coating treatment or packaging, whereas bacterial loads on frozen product remained stable or decreased with time up to 60 d regardless of coating treatment or packaging. Data from the present study indicate that application of edible coatings incorporated with essential oils not only promotes food product safety but also may satisfy the preferences of consumers.

KEYWORDS

antimicrobial edible coating, essential oil, food safety aquaculture, inhibition of *Listeria monocytogenes*, lemongrass

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Aquaculture has been the most rapidly growing global agriculture industry for the past four decades and now accounts for >50% of all fish consumed. Unfortunately, fish is a highly perishable product and has a short shelf life of between 6 and 10 d. *Listeria monocytogenes* has a high prevalence on fish fillets (Feldhusen 2000) and is a serious health concern to humans (Scallan et al. 2011). Thus, numerous methods have been evaluated for antimicrobial activity on fish fillets such as irradiation (Rajkowski 2008), chlorine treatment (Bremer and Osborne 1998), and lactic acid (Bal'a and Marshall 1998); however, these treatments, while effectively reducing bacterial counts, alter the product so that sensory acceptance by consumers was adversely affected.

Hybrid striped bass (HSB), *Morone chrysops × Morone saxatilis*, (also known as sunshine bass) is a commercially important foodfish in the USA and ranks fourth in production at 3,686,477 kg worth US$71 million in 2015 (Anita Kelly, University of Arkansas at Pine Bluff, pers. comm.). While there is a report on storage and sensory quality of HSB fillets during frozen storage (Bett et al. 1998), no research on food safety has been conducted on refrigerated and/or frozen HSB fillets.

Research focusing on using edible films incorporated with natural antimicrobials, such as organic acids, plant extracts, and bacteriocins, has received renewed attention (Cowan 1999; Han 2003; Bakkali et al. 2008; Silva and Fernandes Júnior 2010). Bacteriocins are proteinaceous toxins produced by bacteria, which inhibit growth of similar strains. Nisin is a hydrophobic protein produced by the fermentation of *Lactococcus lactis* and has been shown to have an inhibitory and bacteriostatic effect against gram-positive bacteria (namely *L. monocytogenes, Staphylococcus aureus*, and *Clostridium botulinum*; Nettles and Barefoot 1993). Nisin is a generally recognized as safe (GRAS) compound approved for use in ready-to-eat (RTE) cheese products (Ralph et al. 1995). Hoffman et al. (1998) found nisin to be more stable and remain active for a longer period in corn-zein (CZ) films than in polyethylene films. In a later study, Hoffman et al. (2001) showed that after 48 h, zein films impregnated with nisin reduced cell numbers of *L. monocytogenes* up to 4log.

Essential oils (EOs), one of many secondary metabolites derived from the distillation of plant materials, are also GRAS compounds that have been shown to be effective against food pathogens and spoilage bacteria in both laboratory media and food. Edible coatings incorporated with EOs have been proven to reduce bacterial cell counts of *L. monocytogenes* on sausages (Theivendran et al. 2006), beef (Oussalah et al. 2004; Zinoviadou et al. 2009; Emiroğlu et al. 2010), chicken (Nitzimani et al. 2010), and fish (Gómez-Estaca et al. 2010). The EO of lemongrass (LGEO), *Cymbopogon flexuosus*, has been identified as particularly effective against gram-positive bacteria (Silva and Fernandes Júnior 2010). Lis-Balchin and Deans (1997) confirmed these results when they evaluated 93 different EOs against 20 strains of *L. monocytogenes* and found that LGEO inhibited all 20 strains.

Despite promising results, there is little published research regarding the application of edible coatings infused with EOs. This has been primarily due to the incompatibility between the dosage of EOs required to achieve bacterial inhibition and the resulting change in sensory attributes, making the product unacceptable to the consumer. By introducing EO flavors that are complementary to the food product, a secondary safety step could be created to reduce the occurrence of *L. monocytogenes* in refrigerated RTE food products (Rocourt et al. 2003; FDA 2004). Therefore, this factorial study evaluated the effect of no coating treatment or GRAS compounds, LGEO, and nisin, for reducing *L. monocytogenes* and spoilage bacteria on fresh HSB fillets when incorporated in an edible zein-based coating and stored refrigerated (C) or frozen (−20 C) for various times in one of two packaging types, polyvinyl chloride (PVC) overwrap or vacuum-packaged in barrier bags.

Materials and Methods

Preliminary Work

Preliminary work was conducted on all components of this study. The most effective and

complementary components were selected to be used in the present study. Two coating solutions, and their bases, were tested to determine if they alone imparted any antibacterial effect on microbiological media and the natural flora of tilapia, *Oreochromis niloticus*, fillets or those inoculated with *L. monocytogenes* ATCC-4644. Neither coating nor base inhibited natural flora or *L. monocytogenes* on fish fillets. In addition, coating application method comparing spray versus dipping was evaluated and determined application method had no influence on bacterial inhibition. Therefore, the control used in this study was an uncoated fillet. Concentration of nisin was determined based on Ko et al. (2001). Concentrations of 30,000, 60,000, 90,000, and 120,000 international units (IU)/15 mL of coating solution were tested on microbiological media inoculated with *L. monocytogenes*: 90,000 IU/15 mL of coating solution was found to be most effective, where increased dosage showed no significant gains in inhibition of *L. monocytogenes* ($P < 0.05$). LGEO was selected from 13 plant materials or active compounds tested, including alcohol and water extracts of aloe, sumac, and hops; plant EOs; and dried plant material, due to its inhibitory effect against *L. monocytogenes* and natural fish flora and complementary flavor with fish. Hence, a two inoculation (I) × three coating treatment (C) × two packaging (P) × three storage time factorial design was employed in the current study with respect to *L. monocytogenes* inoculation (No, Yes), coating treatment (control, C; lemongrass essential oil, LG; Nisin, N), packaging (PVC overwrap, PVC; vacuum bag, VAC), and storage time (0, 3, and 5 d, or 0, 30, and 60 d for fresh refrigerated or frozen HSB fillets, respectively). Each factorial trial was repeated twice independent of the first trial in order to build confidence in the collected data.

Bacterial Preparation

Brain-heart infusion (BHI) (Difco Laboratories, Inc., Detroit, MI, USA) slants were inoculated with *L. monocytogenes* strain ATCC-4644 and incubated at 37 C for 48 h. A sample of 60 mL of BHI broth was inoculated with cultured

L. monocytogenes cells and incubated at 37 C for 24 h.

Film Preparation

The edible coating used in this experiment was adapted from Park et al. (2002). Briefly, pharmaceutical and food-grade CZ was acquired from Flo Chemical Corporation (Ashburnham, MA, USA). Two hundred grams of CZ and 60 g of glycerol (99.6%; Acros Organics, Geel, Belgium) were mixed with 2 L of 95% ethyl alcohol and stirred with a magnetic stir bar at medium speed on an Isotemp digital stirring hotplate (Fisher Scientific Co., Fair Lawn, NJ, USA) for 30 min. The pH was adjusted to 5.0 ± 0.02 using 1 N solution of hydrochloric acid (HCl) and measured using an Accument Basic (AB15) pH meter (Fisher Scientific Co.). The coating solution was placed in a 75 C water bath for 30 min and then cooled to room temperature with continuous stirring.

The coating solution was separated into two 2-L glass beakers with 1 L of coating solution per beaker and placed back onto the stir plate at medium speed. To prepare the 8% LGEO coating, 80 mL of 100% food-grade LGEO (LorAnn Oils and Flavors, Lansing, MI, USA) was added to the coating solution and emulsified for 30 sec using a Polytron PT 10-35 (Kinematica AG, Luzern, Switzerland). This final solution was covered and placed on the stir plate until used. The nisin coating solution was prepared by adding 6 g (90,000 IU/15-mL film solution) of nisin (MP Biomedicals, Inc., Solon, OH, USA) to the remaining liter of the coating solution was emulsified and treated as above (Ko et al. 2001). Both coating solutions remained covered and stirred over medium speed until applied to the fish fillets.

Coating Application

All utensils, tables, and instruments were sanitized with Vesphene II or autoclaved before use to ensure no cross-contamination occurred. Application of the coating took place in a walk-in refrigerator (4 C), with cyclical air flow to maintain temperature. A plastic sheet was used to separate the fillets treated with each of

the different coatings. Fresh HSB fillets were obtained from Shuckman's Fish Company and Smokery (Louisville, KY, USA). Fillets were received in vacuum-packaged bags, with five fillets per bag.

Upon arrival, fish fillets were removed from the packages and placed on wire racks, separated by treatment. A volume of 1 L of coating solution was transferred into sanitized, 1-L plastic spray bottles and 500 mL was sprayed over the fillets designated for that treatment. Fillets were allowed to dry for 30 min before being turned over. The remaining 500 mL of coating solution was applied and allowed to dry for another 30 min. Coating solutions were applied one at a time and fillets were weighed before and after coating to determine how much coating solution each fillet received. Control samples that received no coating were packaged first, followed by all noninoculated samples. Noninoculated samples represent natural bacterial flora in the fillet, not that the fillet is sterile. Noninoculated fillets were those to which no *Listeria* was added. Fillets designated for PVC packaging were placed in white foam meat trays (Bauman Paper Co., Lexington, KY, USA) and wrapped with PVC using a HeatSeal LLC model 600A packaging machine (Cleveland, OH, USA). Vacuum-packaged fillets were placed in 10,000 oxygen transfer release bags and sealed with 95% vacuum. All noninoculated samples were stored in a retail display case (4 ± 1 C) and frozen samples were stored in a freezer (−20 C).

Samples to be inoculated with *L. monocytogenes* were placed on sanitized plastic trays and transferred to a refrigerator (4 ± 1 C). Each treatment (control, nisin coating, and 8% LGEO coating) had three replicates per testing day, occurring on Day 0, Day 3, and Day 5 for PVC and on Day 0, Day 30, and Day 60 for frozen samples. Refrigerated and frozen studies were conducted as separate trials that commenced on different days.

Inoculating Procedure

Plastic trays containing the control and coated fillets were taken out of the refrigerator, one treatment at a time, and placed on a sanitized work station. A volume of 1 mL of inoculum was spread dropwise over the surface of each fillet. Using a plastic "hockey stick" applicator, the inoculum was gently spread over the surface of the fillet and great care was taken not to disrupt the coating. After drying for 5 min, inoculated fillets were placed in their designated packaging type; wrapped with PVC or vacuum-packaged in barrier bags with a 95% vacuum. All inoculated samples were stored at 4 ± 1 C.

Microbial Analysis

Both PVC and vacuum-packed samples shared the same Day 0. A lateral strip and two cross-sectional pieces weighing approximately 25 g were removed and weighed into a Labplas 7.5″ × 12″ 4-mil stomaching bag (Labplas, Quebec, Canada). A 1:10 dilution was made with peptone buffer and the sample was pulverized in a stomacher for 1 min. For inoculated samples, dilutions ranging from 10^2 to 10^4 were made into phosphate buffer (pH 7.2). Inoculated control treatments were plated on modified Oxford media (Sigma-Aldrich, St. Louis, MO, USA) using the spread-plate method at a range of dilutions determined during preliminary work. Plates were incubated at 37 C for 48 h. After incubation, plates were counted using a backlit plate counter.

Noninoculated samples were plated onto plate count agar using an Eddy Jet spiral plater (Neutec Group, Inc., Farmingdale, NY, USA). Two serial dilutions were made in phosphate buffer (pH 7.2). A volume of 2 mL of the 10^3 dilution were placed in the spiral plater. Plates were incubated at 20 C for 72 h and enumeration (colony-forming units [CFU]/g) was determined using the Eddy Jet Flash and Go (Neutec Group, Inc.).

Statistical Analysis

Bacterial activity (Log_{10} count) in HSB fillets subjected to the factorial treatments was analyzed by mixed-model factorial ANOVA using PROC MIXED in SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). Differences among least squares means were evaluated using the

DIFF option with the Bonferroni adjustment of P values in SAS in order to apply one of the most conservative means comparisons for reducing experiment-wise error rate. Differences among any responses were declared significant at $P \leq 0.05$ (Zar 1984).

Results

The average weight of refrigerated fillets was 260 g and the average weight of applied coating was 5.0 and 5.7 g for nisin and LGEO treatments, respectively. The average weight of frozen fillets was 259 g, which had a coating weight of 2.3 and 4.1 g for nisin and LEO treatments, respectively.

Refrigerated Samples

Bacterial counts of fresh HSB fillets were significantly different with respect to inoculation, packaging, fillet coating treatment, and time in refrigerated storage (Table 1). Total bacterial counts were greatly reduced when fillets were inoculated with *L. monocytogenes*, independent of packaging, coating treatment, or time in refrigeration ($P < 0.001$ and $P < 0.001$ for Experiment 1 and Experiment 2, respectively). The largest inhibition in bacterial counts occurred in nisin-treated fillets, regardless of other main effects, with LGEO-treated fillets exhibiting intermediate inhibition of bacteria when compared to the control ($P < 0.001$ and $P < 0.001$). Total bacterial counts were significantly lower in PVC-packaged fillets compared to vacuum-packaged fillets in Experiment 1 (4.96 vs. 5.04; $P = 0.037$), but not different in Experiment 2 (5.65 vs. 5.71; $P = 0.361$). Bacterial counts tended to increase with time in refrigerated storage ($P < 0.001$ and $P < 0.001$) but significant interaction ($P < 0.001$ and $P < 0.001$) was detected with respect to time and whether fillets were inoculated. For example, bacterial counts in noninoculated fillets increased or increased and leveled off after 5 d of refrigerated storage (Fig. 1A, C) whereas few differences in bacterial counts were noted with respect to time in storage of inoculated fillets (Fig. 1B, D).

Significant two- and three-way interactions were detected among main effects (Table 1),

but few were consistent between replicate experiments (Table 1; Fig. 1). For example, among noninoculated fillets (Fig. 1A, C), bacterial counts in LGEO-treated fillets were lower than or comparable to bacterial counts in nisin-treated fillets, whereas among inoculated fillets (Fig. 1B, D), bacterial counts in nisin-treated fillets were significantly lower than those in control or LGEO-treated fillets. Moreover, in inoculated fillets (Fig. 1B, D), counts of *L. monocytogenes* in LGEO-treated fillets were only slightly lower than those in the control (no coating) fillets. Second, in Experiment 1 noninoculated fillets (Fig. 1A), bacterial counts were greater in PVC-packaged fillets than in vacuum-packaged fillets for the control and nisin treatments, regardless of time in storage. However, the opposite was true for LGEO-treated fillets: bacterial counts in PVC-packaged fillets ($\bar{y} = 7.28$) were less than counts found in vacuum-packed fillets ($\bar{y} = 7.66$). On the other hand, in Experiment 2, noninoculated fillets (Fig. 1C), this pattern with respect to LGEO-treated fillets was flipped, that is, bacterial counts in PVC-packaged LGEO fillets ($\bar{y} = 8.15$; $t = 5$ d) were greater than counts found in vacuum-packed LGEO fillets ($\bar{y} = 7.69$; $t = 5$ d).

Frozen Samples

Bacterial counts in frozen HSB fillets were significantly different with respect to inoculation, packaging, fillet coating treatment, and time in frozen storage with no meaningful interactions (Table 2). Total bacterial counts were somewhat reduced when frozen fillets were inoculated with *L. monocytogenes*, independent of packaging, coating treatment, or time in frozen storage ($P < 0.001$ and $P < 0.001$ for Experiment 1 and Experiment 2, respectively). The lowest bacterial counts were measured in nisin-treated frozen fillets, regardless of other main effects, whereas LGEO-treated fillets exhibited intermediate bacterial counts when compared to those in the control and nisin treatments ($P < 0.001$ and $P < 0.001$). Bacterial counts were higher in vacuum-packaged frozen fillets compared to PVC-wrapped fillets

TABLE 1. *Bacterial activity (Log$_{10}$ count) of corn-zein-based edible coating on refrigerated hybrid striped bass (HSB) fillets with respect to* Listeria monocytogenes *inoculation (no, yes), coating treatment (C = control; LG = lemongrass essential oil; N = Nisin), packaging (PVC = PVC overwrap; VAC = vacuum bag), and time in storage (0, 3, and 5 d).[1]*

Inoculated	Treatment	Packaging	Days	Exp. 1[2] activity	Exp. 2[2] activity
No	C	PVC	0	5.49b	5.80b
No	C	PVC	3	7.88a	8.84a
No	C	PVC	5	8.35a	8.68a
No	C	VAC	0	5.49b	5.80b
No	C	VAC	3	7.70a	8.68a
No	C	VAC	5	8.20a	8.59a
No	LG	PVC	0	4.75c	5.07b
No	LG	PVC	3	5.93b	7.94a
No	LG	PVC	5	7.28a	8.15a
No	LG	VAC	0	4.75c	5.07b
No	LG	VAC	3	6.56b	8.10a
No	LG	VAC	5	7.66a	7.69a
No	N	PVC	0	5.18c	4.99b
No	N	PVC	3	7.37b	7.80a
No	N	PVC	5	8.15a	8.34a
No	N	VAC	0	5.18b	4.99b
No	N	VAC	3	7.35a	8.29a
No	N	VAC	5	7.68a	8.10a
Yes	C	PVC	0	5.11	5.89
Yes	C	PVC	3	4.68	5.73
Yes	C	PVC	5	4.65	5.34
Yes	C	VAC	0	5.11	5.89
Yes	C	VAC	3	4.92	5.67
Yes	C	VAC	5	4.94	5.75
Yes	LG	PVC	0	4.90a	5.50
Yes	LG	PVC	3	4.01b	4.71
Yes	LG	PVC	5	3.78b	4.41
Yes	LG	VAC	0	4.90a	5.50
Yes	LG	VAC	3	4.01b	5.15
Yes	LG	VAC	5	4.23b	5.07
Yes	N	PVC	0	1.00	1.45
Yes	N	PVC	3	1.00	1.49
Yes	N	PVC	5	1.23	1.09
Yes	N	VAC	0	1.00	1.45
Yes	N	VAC	3	1.00	1.63
Yes	N	VAC	5	1.52	1.74
Pooled SEM				0.12	
Main effect means[3]					
No				6.63a	7.26a
Yes				3.36b	4.10b
	C			5.96a	6.70a
	LG			5.15b	6.01b
	N			3.89c	4.32c
		PVC		4.96b	5.65
		VAC		5.04a	5.71
			0	4.32c	4.76b
			3	5.12b	6.15a
			5	5.55a	6.12a

TABLE 1. *Continued*

Inoculated	Treatment	Packaging	Days	Exp. 1[2] activity	Exp. 2[2] activity
ANOVA Source, Pr > F					
Inoculated (I)				< 0.001	< 0.001
Treatment (T)				< 0.001	< 0.001
Packaging (P)				0.037	0.361
Days (D)				< 0.001	< 0.001
I × T[4]				< 0.001	< 0.001
I × P[4]				0.119	0.163
I × D[4]				< 0.001	< 0.001
T × P[4]				0.012	0.799
T × D[4]				< 0.001	0.019
P × D[4]				0.321	0.611
I × T × P[5]				0.023	0.324
I × T × D[5]				< 0.001	0.532
I × P × D[5]				0.020	0.161
T × P × D[5]				0.263	0.516
I × T × P × D				0.149	0.839

[1] Values are least squares means of $n = 3$ fillets per treatment combination.

[2] Results of two independent replicate experiments (Experiment 1 and Experiment 2).

[3] Main effect least squares means in the same column with different letters are different ($P \leq 0.05$).

[4] See Figure 1 for depiction of two-way interactions.

[5] In the case of significant three-way interaction, means with different letters with respect to days in storage within an inoculation (I) × coating treatment (T) × packaging (P) level are different ($P \leq 0.05$).

in both replicate experiments ($P < 0.001$ and $P < 0.013$). In Experiment 1, bacterial counts increased at 30-d frozen storage, then decreased at 60 d to levels less than those measured at Day 0 ($P < 0.001$). In Experiment 2, bacterial counts slightly decreased ($P = 0.003$) with storage time.

Statistically significant two- and three-way interactions were detected among main effect factors (Table 2); however, no consistent trends could be discerned, which was corroborated by few significant trends with respect to frozen storage time among the 12 I × T × P treatment combinations (Table 2; Fig. 2). For example, for Experiment 1, noninoculated fillets (Fig. 2A), bacterial counts in nisin-treated, vacuum-packaged fillets rose to higher levels than those in the control and LGEO treatments at Day 30 but decreased to levels comparable to the other treatments by Day 60; in Experiment 2 for this treatment combination (Fig. 2C), bacterial counts in nisin and LGEO noninoculated fillets were lower than the control regardless of packaging and remained lower and unchanged for the 60 d of frozen storage. Moreover, LGEO, as well as nisin, performed in inhibiting bacterial

growth in Experiment 2 noninoculated frozen fillets (Fig. 2C). Among inoculated frozen fillets (Figs. 2B, D), nisin-treated fillets consistently exhibited the lowest bacterial counts, with LGEO-treated fillets showing strongly intermediate inhibition compared to the control and nisin treatments. There was a statistically significant drop, and then increase, in bacterial counts in Experiment 1 for inoculated frozen fillets treated with LGEO and then wrapped in PVC (Fig. 2B) that was not found in Experiment 2 (Fig. 2D).

Discussion

This is the first report on the use of LGEO on fresh HSB fillets during refrigerated and frozen storage. Both nisin and LGEO treatments were found to reduce bacterial cell counts in fresh and frozen HSB fillets stored under refrigerated and frozen storage conditions packaged in either PVC or under vacuum. *L. monocytogenes* is a poor competitor with spoilage bacteria occurring on the surface of fresh meat; however, in the present study, total viable counts (TVCs) of *L. monocytogenes* remained unchanged in
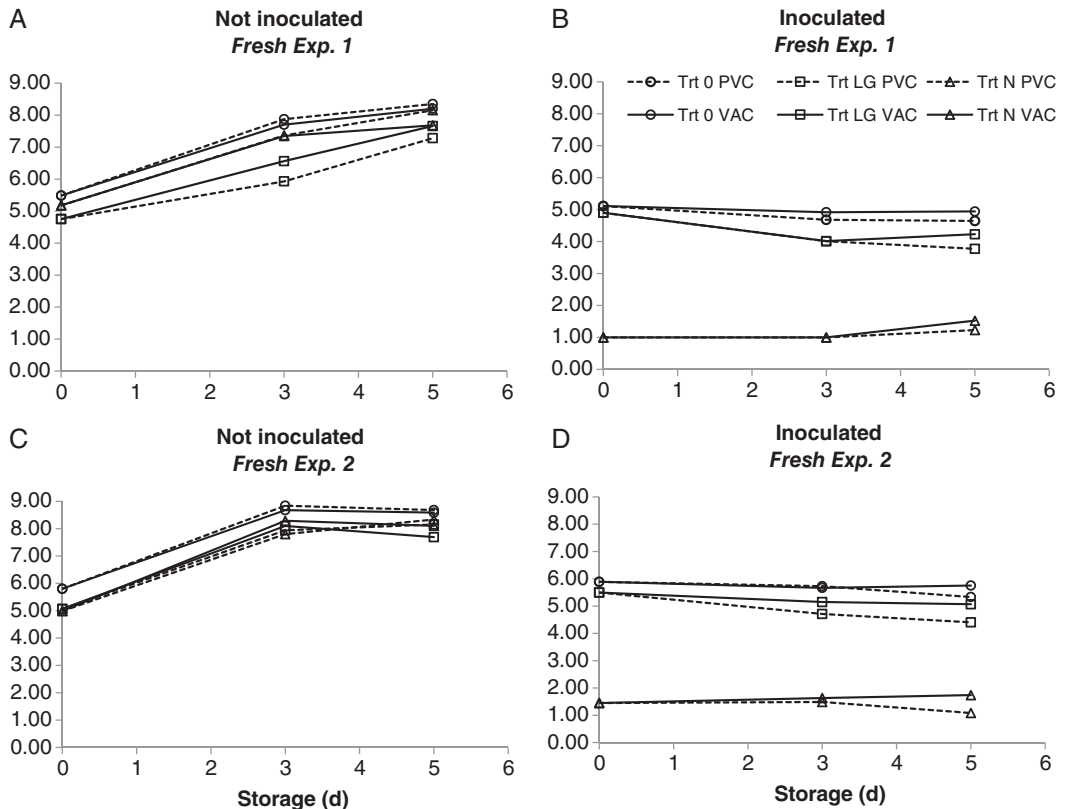
FIGURE 1. *Bacterial activity (Log$_{10}$ count) of corn-zein-based edible coating on fresh refrigerated hybrid striped bass (HSB) fillets with respect to* Listeria monocytogenes *inoculation (no – Panel A and C; yes – Panel B and D), time in storage (0, 3, and 5 d), packaging (PVC overwrap [PVC] – dashed line; vacuum bag [VAC] – solid line), and coating treatment (control [Trt 0] – circle; lemongrass essential oil [LG] – square; Nisin [N] – triangle). Values are least squares means of* n = 3 *fillets per treatment combination for two replicate experiments.*

the presence of bacterial loads exceeding 10^8, indicating that viable cells can remain intact despite the presence of other spoilage bacteria. Nisin, a known bacteriocin with antilisterial properties, showed both bacteriocidal and bacteriostatic effects on HSB fillets inoculated with *L. monocytogenes* when incorporated into a CZ coating. Although less effective, LGEO had a marked and prolonged effect on *L. monocytogenes* under the same packaging and storage conditions.

Although the specific native bacteria were not individually identified, Gram and Dalgaard (2002) reported that the gram-negative bacteria *Pseudomonas* spp. and *Shewanella* spp. are typically responsible for spoilage of refrigerated fish fillets and that gram-positive bacteria,

lactic acid bacteria, and *Corynebacterium* spp. generally spoil frozen, vacuum-packaged fillets. Gram-positive bacteria have been found to be particularly susceptible to nisin. The fact that nisin inhibited spoilage bacteria in the present study on both packaging types in HSB fillets, whether refrigerated or frozen, suggests the predominant bacteria responsible for spoilage of HSB fillets were gram-positive. Toxicity of nisin to these organisms results from its action on the cytoplasmic membrane, resulting in disruption of ion transport in the cell and membranes, inhibition of respiratory activity and hydrolysis, and partial efflux of adenosine triphosphate (Abee et al. 1994). The action of nisin on the cell membrane results in the formation of pore-like structures that allow

TABLE 2.   *Bacterial activity (Log$_{10}$ count) of corn-zein-based edible coating on frozen hybrid striped bass (HSB) fillets with respect to* Listeria monocytogenes *inoculation (no, yes), coating treatment (C = control; LG = lemongrass essential oil; N = Nisin), packaging (PVC = PVC overwrap; VAC = vacuum bag), and time in storage (0, 30, and 60 d).*[1]

Inoculated	Treatment	Packaging	Days	Exp. 1[2] activity	Exp. 2[2] activity
No	C	PVC	0	5.50	5.23
No	C	PVC	30	6.40	5.70
No	C	PVC	60	5.58	5.56
No	C	VAC	0	5.50	5.23b
No	C	VAC	30	6.40	7.08a
No	C	VAC	60	5.58	6.58ab
No	LG	PVC	0	5.60	4.34
No	LG	PVC	30	5.81	3.88
No	LG	PVC	60	4.55	3.00
No	LG	VAC	0	5.60	4.34
No	LG	VAC	30	6.79	3.78
No	LG	VAC	60	4.63	3.44
No	N	PVC	0	5.56a	3.91
No	N	PVC	30	5.73a	3.34
No	N	PVC	60	3.93b	3.00
No	N	VAC	0	5.56b	3.91
No	N	VAC	30	9.03a	3.69
No	N	VAC	60	4.67b	4.01
Yes	C	PVC	0	5.61	5.48
Yes	C	PVC	30	5.24	5.53
Yes	C	PVC	60	5.13	5.27
Yes	C	VAC	0	5.61	5.48
Yes	C	VAC	30	5.57	5.45
Yes	C	VAC	60	5.38	5.26
Yes	LG	PVC	0	4.95a	4.53
Yes	LG	PVC	30	3.12b	3.95
Yes	LG	PVC	60	3.92ab	3.39
Yes	LG	VAC	0	4.95	4.53
Yes	LG	VAC	30	4.14	4.11
Yes	LG	VAC	60	4.12	3.68
Yes	N	PVC	0	1.01	2.52
Yes	N	PVC	30	1.06	2.01
Yes	N	PVC	60	1.07	2.04
Yes	N	VAC	0	1.01	2.52
Yes	N	VAC	30	1.19	1.29
Yes	N	VAC	60	1.18	2.26
Pooled SEM				0.28	0.36
Main effect means[3]					
No				5.80a	4.45a
Yes				3.68b	3.85b
	C			5.73a	5.65a
	LG			4.96b	3.91b
	N			3.52c	2.88c
		PVC		4.54b	4.04b
		VAC		4.94a	4.26a
			0	4.81b	4.33a
			30	5.15a	4.15ab
			60	4.25c	3.96ba

TABLE 2. *Continued*

Inoculated	Treatment	Packaging	Days	Exp. 1[2] activity	Exp. 2[2] activity
ANOVA Source, Pr > F					
Inoculated (I)				< 0.001	< 0.001
Treatment (T)				< 0.001	< 0.001
Packaging (P)				< 0.001	0.013
Days (D)				< 0.001	0.003
I × C[4]				< 0.001	< 0.001
I × P[4]				0.055	0.008
I × D[4]				< 0.001	0.045
T × P[4]				0.020	0.402
T × D[4]				< 0.001	< 0.001
P × D[4]				< 0.001	0.064
I × T × P[5]				0.002	0.107
I × T × D[5]				< 0.001	0.095
I × P × D[5]				0.061	0.158
T × P × D[5]				0.049	0.372
I × T × P × D				0.008	0.558

[1]Values are least squares means of $n = 3$ fillets per treatment combination.

[2]Results of two independent replicate experiments (Experiment. 1 and Experiment 2).

[3]Main effect least squares means in the same column with different letters are different ($P \leq 0.05$).

[4]See Figure 2 for depiction of two-way interactions.

[5]In the case of significant three-way interaction, means with different letters with respect to days in storage within an inoculation (I) × treatment (T) × packaging (P) level are different ($P \leq 0.05$).

hydrophilic, low-molecular-weight constituents to leak out.

Plant oils and extracts have been used in foods as natural preservatives in raw products (Nychas 1995) and are recognized to contain natural antimicrobial activity. They include extracts from tea, turmeric, shallots, onion, and garlic and EOs from thyme, oregano, and sage. Extracts from oregano, thyme, rosemary, clove, and sage have been used to improve sensory characteristics and extend shelf life in foods, and several have been reported to have antimicrobial properties (Burt 2004). These antimicrobial properties are due to the phenolic compounds present in the EO (carvacrol, thymol, and terpenes) and have been used to extend shelf life of fresh fish (Goulass and Kontominas 2007; Giatrakou et al. 2008; Atrea et al. 2009; Kykkidou et al. 2009; Pyrgotou et al. 2010).

In the present study, LGEO incorporated into an edible CZ coating successfully inhibited *L. monocytogenes* on fresh, refrigerated HSB fillets when stored in PVC and vacuum packaging. In the only other published report on the use of LGEO on fish fillets, Ahmad et al. (2012) stated that sea bass, *Lates calcarifer*, slices wrapped in a gelatin film and coated with LGEO exhibited reduced TVC by approximately 2-log CFU/g after 12 d of storage. Sea bass fillet slices coated with LGEO and wrapped in a gelatin film had 5.6 CFU/g after 12 d of storage, compared to slices not wrapped in a gelatin film (7.9 CFU/g after 12 d of storage), and slices wrapped in a gelatin film but without LGEO had 7.0 CFU/g after 12 d of storage. Pezeshk et al. (2011) reported that use of turmeric and shallot extracts reduced TVC in vacuum-packaged rainbow trout, *Oncorhynchus mykiss*, fillets and extended shelf life 17 d compared to fillets without the extracts. Similarly, Pyrgotou et al. (2010) reported that fresh rainbow trout fillets treated with 0.2 and 0.4% oregano EO and stored under modified atmosphere packaging (MAP) had significantly lower TVC compared to fillets not treated with oregano EO. Further, addition of oregano EO extended the shelf life of the fillets by 7 d. Coating swordfish, *Xiphias gladius*, fillets with 0.1% oregano EO, and stored under aerobic packaging or MAP, reduced TVC and extended shelf life by 8 d compared to fillets stored using
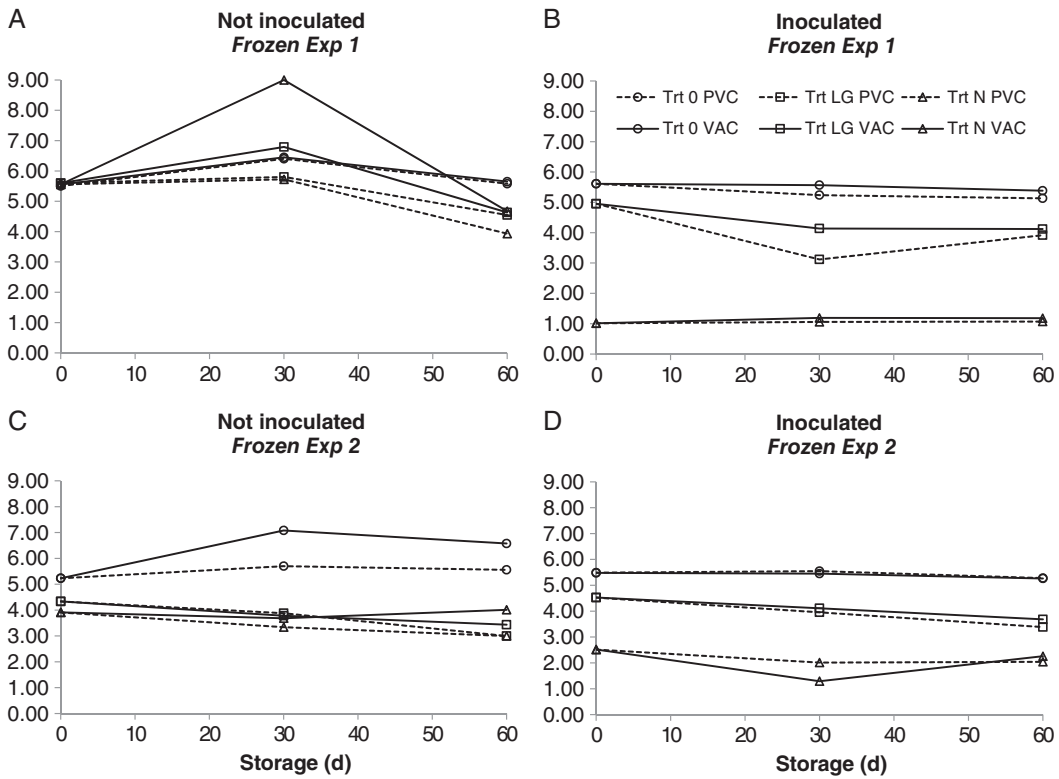
FIGURE 2. *Bacterial activity (Log₁₀ count) of corn-zein-based edible coating on frozen hybrid striped bass (HSB) fillets with respect to* Listeria monocytogenes *inoculation (no – Panel A and C; yes – Panel B and D), time in storage (0, 30, and 60 d), packaging (PVC overwrap [PVC] – dashed line; vacuum bag [VAC] – solid line), and coating treatment (control [Trt 0] – circle; lemongrass essential oil [LG] – square; Nisin [N] – triangle). Values are least squares means of* n = 3 *fillets per treatment combination for two replicate experiments.*

aerobic packaging (Giatrakou et al. 2008). However, MAP packaging imparts its own antimicrobial effect of CO_2 on aerobic spoilage bacteria (*Pseudomonas*). In the present study, fillets were only vacuum packaged and no MAP was utilized so that results were not confounded by the use of MAP.

In the current study, bacterial counts in vacuum-packaged samples were slightly higher than in PVC-packaged samples for both refrigerated and frozen fillets, but the magnitude of the difference was small, on the order of 0.06–0.08log in refrigerated fillets and 0.22–0.4log in frozen fillets. Interestingly, bacterial counts in the refrigerated LGEO-coated fillets decreased with time in both PVC- and vacuum-packaged samples when compared to bacterial counts in the control samples when

samples were inoculated with *L. monocytogenes* whereas the opposite was true when samples were not inoculated. This indicates that packaging conditions, in combination with other factors, can impact the ability of antibacterial components to be released from the coating material. Vacuum packaging is widely used in the food industry to keep food fresh because of its low cost and its effect of reducing oxidative reactions. It can also be used in conjunction with other methods of preservation, including low temperature storage, antioxidants, and use of antimicrobial agents, to delay spoilage, extend shelf life, improve food safety, and maintain high-quality products for the consumer.

The precise mode of action of EOs against food pathogens largely depends on the active

components. It is suggested that the EOs, which contain a large number of terpenoids, affect the bacterial cells' cytoplasmic membrane, where hydrophobic interactions between lipids and proteins are disrupted, causing increased permeability and loss of cellular components (Helenius and Simons 1975; Franklin and Snow 2000). The antimicrobial activity of LGEO is due to alpha-citral (geranial) and beta-citral (neral) components that exhibit bacteriocidal action on both gram-negative and gram-positive bacteria (Onawunmi et al. 1984). Further, cyclic terpenoids, such as limonene and borneol, are found in LGEO at concentrations of 4.4 and 2.2%, respectively (Sikema et al. 1994). These active components accumulate in the bacterial cell membrane, causing loss of integrity and disruption of the proton motive force.

Similar mechanisms have been suggested regarding the action of citral, a terpenoid whose isomers can be found at concentrations of up to 72% in LGEO (Tzortzakis and Economakis 2007). Tamir et al. (1984) identified citral as causing cellular damage to *L. monocytogenes*, affecting the membrane through lipid peroxidase mechanisms, though its analogues (including geraniol and neral) had no effect when tested independently. This suggests that effectiveness is likely due to a combination of active components. Burt (2004) stated that EO compounds can have an additive, a synergistic, or an antagonistic effect on bacterial inhibition. Whole EOs have been found to be more effective than singular, major components, indicating that minor components may aid in antimicrobial activity (Gill et al. 2002; Mourey and Canillac 2002). Carvacrol, a major and well-studied component of oregano EO, has a synergistic effect with its precursor, *p*-cymene. It is a weak antibacterial component that causes a greater swelling of the bacterial cell than carvacrol alone. When used in combination, *p*-cymene allows carvacrol to be more easily transported into the cell (Ultee et al. 2000).

It has been reported that various strains of *L. monocytogenes* have different sensitivities to various EOs. Desai et al. (2012) found that serotypes 1/2b and 4c were not sensitive to lemon EOs and two serotypes (3b and 4b) were only moderately sensitive; however, when they used a 5% lemon EO solution, it was effective in reducing all four serotypes, as well as TVC in raw channel catfish, *Ictalurus punctatus*, fillets. In the present study, LGEO was obtained from a commercial source and is readily available. No attempt was made to investigate the composition of the EO to determine antimicrobial agents present. As the antimicrobial activity of an EO depends on its composition, it could be affected by agronomic, seasonal, geographic, and genetic factors and extraction method. These differences could offer an explanation as to why LGEO had antimicrobial properties in the present study but that citrus EOs were inconsistently effective in reducing *Listeria* and TVC in catfish fillets.

The adaptation by *L. monocytogenes* that allows growth at low temperatures is accomplished by increasing the proportion of unsaturated fatty acyl chain of lipids in the cell membrane (Abee et al. 1994). The ability of citral and other cyclic terpenes to directly disrupt lipid bonds may explain why *Listeria* is particularly susceptible to LGEO. Nisin disrupts the bacterial cell wall of *L. monocytogenes* within 20 sec of contact, measured by a loss of K^+ from the cells (Thomas and Wimpenny 1996). In the present research, an immediate and sustained reduction of *L. monocytogenes* numbers was observed in both PVC- and vacuum-packaged samples. The effectiveness of nisin has been documented to increase in foods with higher salt concentrations and decrease when exposed to low pH (4.0–5.0) (Abee et al. 1994; Thomas and Wimpenny 1996). Although pH of the CZ coating was adjusted to 5.0, it did not seem to have an adverse effect on the action of nisin against *Listeria*.

Conclusions

The use of GRAS compounds to alleviate risk of illness resulting from food pathogens on RTE products has great potential. Although nisin has proven to be an effective bacteriocin against *Listeria* in meat, dairy, and canned food, its role as a staple additive in the food sector is uncertain. Foremost, nisin is an expensive additive and the cost has limited its use to a wider variety of

products. Second, obtaining a consistent product from overseas has been riddled with challenges due to varying regulations and standards for production that vary between countries.

Exploring the use of natural, readily available compounds, such as EOs, is becoming an attractive alternative. Although a wealth of knowledge potentiating the use of EOs in food protection is available, the lack of real-world application is evident. This study bridges this gap and provides a clear path for further research. Future work in this field should focus on the physiochemical properties of LGEO coatings, potential chemical or sensory changes to the food product, verification of the safe use and dosage of LGEO, and the potential for commercialization. Although challenges exist, data from the present study indicate that application of edible coatings incorporated with EOs promotes not only food product safety but also may satisfy the environmental conscience of the consumer.

Acknowledgments

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